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Original Research Article

Adicardin and Other Coumarins from *Breonadia salicina* (Vahl) Hepper

Barnabas J. Nvau[1]*, Bawazeer Sami[2], Olugbenga S. Ajibade[3], Irvine A. Gray[4], John. O. Igoli[4, 5]

[1]*Department of Chemistry, Plateau State University, Bokkos, Nigeria.*
[2]*College of Pharmacy, Umm Al-qura University, Makkah, Saudi Arabia,*
[3]*Department of Chemical Sciences, Redeemer's University, Ede, Osun State, Nigeria.*
[4]*Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, 161 Cathedral Street, G4 0RE, Glasgow UK.*
[5]*Department of Chemistry, University of Agriculture, Makurdi, Nigeria.*

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ABSTRACT

Breonadia salicina is an ethnomedicinal plant used in North Central Nigeria to treat sleeping sickness and respiratory diseases. The stem bark of the plant was extracted with methanol and the extract partitioned between dichloromethane and water. The dichloromethane soluble portion was subjected to column chromatography which resulted in the isolation of 7-(*β*-D-apiofuranosyl (1-6)-*β*-D-glucopyranosyl) umbelliferone, *α*-amyrin, stigmasterol, 7-hydroxycoumarin and 6- hydroxy-7-methoxy coumarin. The structures of these compounds were elucidated based on NMR spectral analysis. This is the first report of these compounds from the plant.

Keywords: Terpenoids, Coumarins, Medicinal plant, *Breonadia salicina*, Adicardin.

Introduction

The maintenance of human and animal health has been a major challenge since time immemorial. Man then resorted to the use of medicinal plants as a source of relief for health challenges.[1] Although man's advance in science has produced single or combined chemical entities for the treatment of various animal and human diseases, there is still a heavy reliance on medicinal plants to treat many illnesses.[2, 3] The high cost of modern medicine and resistance of most organisms to these drugs are on the increase, and this has led to the high interest in medicinal plants.[4] Traditional medicine or ethnobotany is believed to be cheap and can provide novel drug scaffolds for drug discovery.[5-7]*Breonadia salicina* (Rubiaceace) is a monotypic genus of flowering plants. It is widely distributed in Mali, Benin, Ethiopia, Yemen, Saudi Arabia, Madagascar and Nigeria.[4] They grow on river banks. The plant is widely used to treat cancer, gastrointestinal illness, fever, headaches, arthritis, diabetes, inflamed wounds, ulcers, respiratory and fungal infections.[4,8] In addition the stem bark of *B. salicina* is used to treat diarrhea and other stomach/digestive problems.[4, 9]Al-qurainy*et al* 2013, reported that the methanol extract showed great activities on *Bacillus subtilis, Pseudomonas aeruginosa, Shigella sonnei, Escherichia coli and Staphylococcus aureus*.[4] The plant extracts also showed high mortality rate on *trypanosoma brucei*.[8] Phytochemical screening of the plant extract showed the presence of saponins, alkaloid, tannins, anthraquinones, flavonoids, glucosides and ursolic acid.[8,10] There are scanty reports on the plant's secondary metabolites which may be responsible for its biological activities. This report is on the extraction, isolation and elucidation of the secondary metabolites from the stem bark of the plant.

*Corresponding author. E mail: johnnvau5@gmail.com
Tel: +2348035807728

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Materials and Methods

General experimental procedures

^{1}H-NMR spectra were acquired on a Bruker 400 MHz spectrophotometer using deuterated DMSO and the chemical shifts are reported in ppm with respect to TMS. All solvents except NMR solvents were distilled before use. Silica gel (120–230 mesh) was used for column chromatography and pre-coated TLC plates (Merck, Germany) were used for monitoring the column fractions.

Plant source and Collection

The stem barks of *Breonadia salicina* were collected at Mushere, Bokkos Local Government Area of Plateau State Nigeria in February 2018. The plant sample was authenticated at Herbarium section Herbarium No: FHJ257) of the Federal School of Forestry Jos, Nigeria. The fresh stem bark collected were dried under shade for three weeks.

Extraction and isolation

The air dried and powdered stem bark (1.0 kg) was macerated in 2.0 L methanol for three days and then filtered. The extract was concentrated on a rotary evaporator at reduced pressure and temperature. A portion (10 g) of the solvent free extract was dissolved in 20 mL of methanol and then treated with hexane/water (80/20). This process was repeated five times and pooled into one fraction. The aqueous portion of the extract was dried on a rotary evaporator to remove any residual hexane. The aqueous portion was further treated with dichloromethane (DCM). The process was repeated five times and fractions collected were pooled into a single fraction. The hexane and dichloromethane fractions were dried using rotary evaporator to yield 1.90 g and 3.20 g of hexane and dichloromethane extracts, respectively. A portion (1.0 g) of the dichloromethane extract was dissolved in 20 mL of DCM and adsorbed with 30 g of silica gel. The dried admixture was introduced on a column packed with 100 g of silica gel in hexane. The column was eluted gradient-wise with 300 ml of 100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90 hexane in ethyl acetate and 100:0, 90:10 and 80:20 mixtures of ethyl acetate in methanol. A total of 180 fractions of 20 mL each were collected and monitored by TLC. Fractions F37, F38, F39-45 eluted with 20% ethyl acetate in hexane and fractions F162, F167, F169, F170, F172, F174 eluted with 10% methanol in ethyl acetate showed

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single spots on TLC. These fractions were allowed to stand and the solid precipitates produced were subjected to NMR analysis.

Results and Discussion

Phytochemical investigation of the dichloromethane soluble fraction of the methanol extract of the stem bark of *B. salicina* afforded five compounds (**1**-**5**). The structures of the isolated compounds were assigned based on signals in their ^{1}H- and ^{13}C-NMR and confirmed by correlations in their 2D (COSY, HMBC, HMQC) NMR and comparison with literature reports.[11,12]The ^{1}H-NMR spectrum of compound **1** indicated three sets of doublets at δ_H 6.32, 7.65, 7.99 and a multiplet at δ 7.03, typical of a C-7 substituted coumarin skeleton.[13] The spectrum indicated two anomeric protons at 4.81 and 4.98 suggesting the other signals within the range of 3.20 to 3.95 are for sugar moieties. From the COSY spectrum, the olefinic proton at δ 6.32 (J = 9.5 Hz) was coupled to another one at δ 7.99 (J = 9.5 Hz) while an aromatic proton at δ 7.65 was coupled to a proton δ 7.03 indicating an ortho coupling from an ABX substituted aromatic ring as there was a meta coupled aromatic doublet at 7.04 ppm. The cis-coupling of the olefinic protons further supported a coumarin skeleton.

The ^{13}C-NMR spectrum showed 20 signals made up of fivequaternaries (one carbonyl), twelve methine and three methylene carbons. From the HSQC spectrum, protons at δ_H 6.32, 7.99, 7.65, 7.03 and 7.04 correlated to the carbons at 113.7, 144.7, 130.0, 113.9 and 103.8, respectively indicating their points of attachment while the anomeric proton at 4.98 was attached to the carbon at δ_C 100.4, and δ_H 4.80 to δ_C 109.8 ppm. In the HMBC spectrum, the correlations of H-3 to C-2 and C-4a, H-4 to C-2, C-5 and C-8a, H-5 to C-4, C-7 and C-8a, H-6 to C-4a and C-8 confirmed the coumarin skeleton. The anomeric proton at δ 4.98 showed correlations to C-7, C-2' and C-5' which supported the substitution of the coumarin ring at position C-7. The HMBC spectrum was also used to establish the sugar moieties. These spectral features are in close agreement to that published for adicardin.[11=14]In addition, a survey of the literature showed that this compound has been isolated from *Gmelina arborea*[11] and *Phlojodicarpus villous.*[12] However, this is the first time that this compound is being isolated from *Breonoida salicicna.* Other compounds isolated were α-amyrin (**2**), a mixture of stigmasterol (**3a**) and sitosterol (**3b**), 7-hydroxy coumarin (**4**) and 6- hydroxy-7-methoxy coumarin (**5**).[15-18]

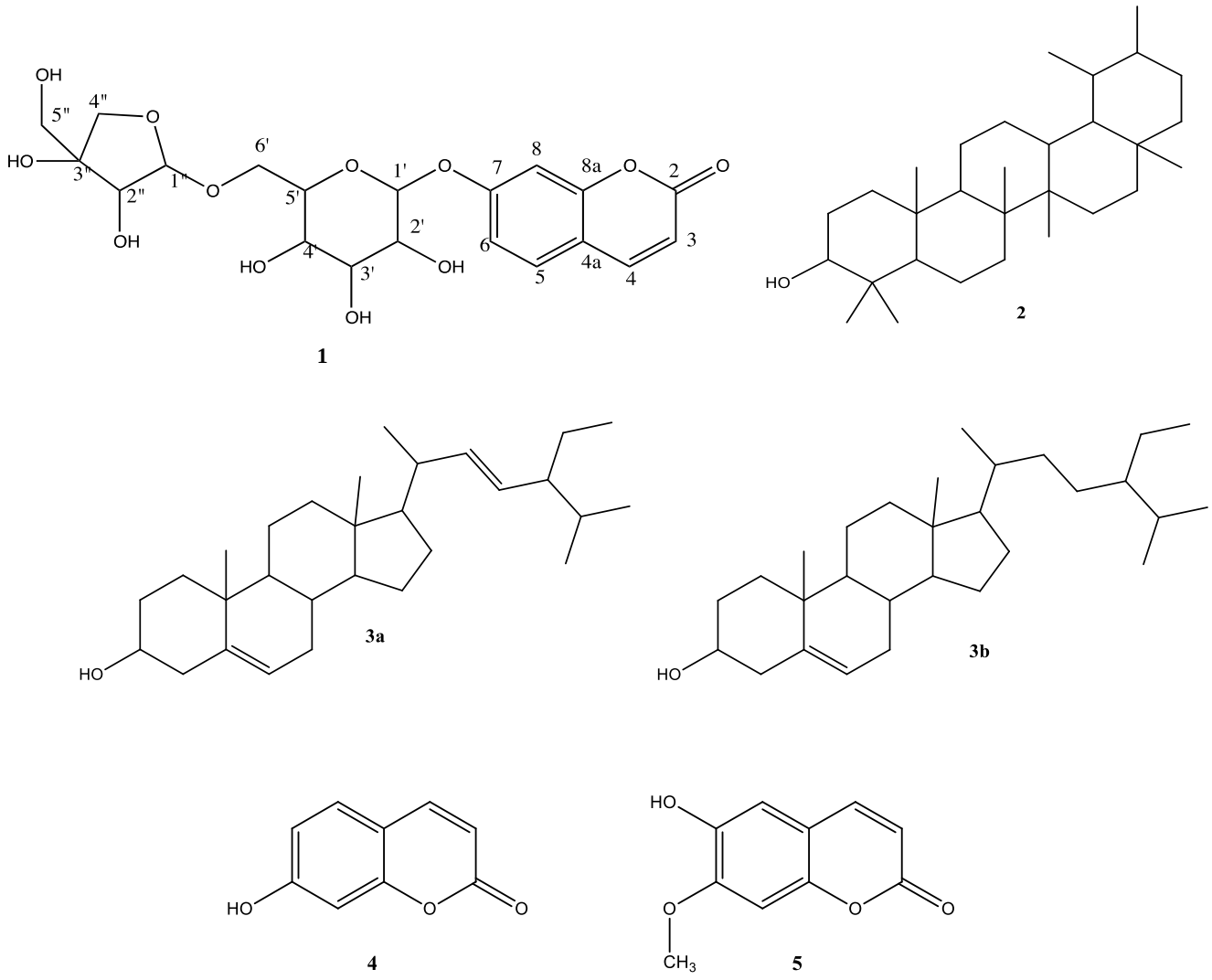

Figure 1: Chemical Structures of Compounds 1 – 5.

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Table 1: ^{1}H- and ^{13}C-NMR data for Adicardin (**1**), in DMSO-*d6* in comparison with literature data

	Compound 1		Literature (Li *et al* 2009)	
Position	**$\delta_H$mult, (*J* in Hz)**	**δ_C**	**$\delta_H$mult, (*J* in Hz)**	**δ_C**
1	-	-	-	-
2	-	160.7	-	160.2
3	6.32 (d, 1H, *J* = 9.5Hz)	113.4	6.32 (d, 1H, 9.2Hz)	113.2
4	7.99 (d, 1H, *J* = 9.5Hz)	144.3	7.99 (d, 1H, 9.2Hz)	144.2
4a	-	113.5	-	114.3
5	7.65 (d, 1H, *J* = 9.3Hz)	129.5	7.64 (d, 1H, 8.2Hz)	129.5
6	7.02 (m, 1H)	113.9	7.02–7.00 (m, 1H)	113.4
7	-	160.6	-	160.1
8	7.02 (m, 1H)	103.8	7.02–7.00 (m, 1H)	103.3
8a	-	155.5	-	155.0
1’	4.98 (d, 1H, *J* = 6.4Hz)	100.0	4.98 (d, 1H, *J* = 7.2Hz)	99.9
2’	3.74 (dd, 1H, *J* = 6.2, 3.1Hz)	75.4	3.73 (dd, 1H, *J* = 6.4,3.2Hz)	75.5
3’	3.59 (d, 1H, *J* = 9.2 Hz)	73.1	3.59-3.57 (1H)	73.1
4’	3.59 (d, 1H, *J* = 9.2 Hz)	69.9	3.59-3.57 (1H)	69.9
5’	3.45 (dd, 1H, *J* = 11.2, 7.0Hz)	76.5	3.44 (dd, 1H, *J* = 11.2, 3.2Hz)	76.4
6’	3.13 (td, 2H, *J* = 9.9, 5.1Hz)	67.8	3.16-3.11 (2H)	67.6
1”	4.81 (1H)	110.1	4.79 (d, *J* = 3.6Hz)	109.8
2”	3.90 (d, 1H, *J* = 9.4Hz)	76.0	3.88 (d, 1H, *J* = 9.2Hz)	75.9
3”	-	76.2	-	75.9
4”	3.29 (m, 2H)	75.6	3.30-3.25 (2H)	75.5
5”	3.38 (dd, 2H, *J* = 11.2, 5.5 Hz)	63.4	3.39-3.31 (2H)	63.2
2’-OH	5.41 (d, *J* = 4.8 Hz)	-	5.40 (d, 4.8Hz)	-
3’-OH	5.17 (t, *J* = 5.6 Hz)	-	5.18 (t, 5.2Hz)	-
3”-OH	5.17 (t, *J* = 5.6 Hz)	-	5.18 (t, 5.4Hz)	-
2”-OH	5.01 (d, *J* =7.3 Hz)	-	5.01 (d, 7.2Hz)	-
4’-OH	4.71 (d, *J* = 5.6 Hz)	-	4.71 (t, 6.0Hz)	-
5”-OH	4.47 (s)	-	4.46 s	-

Conclusion

Adicardin, a rare substituted coumarin with two sugar moieties has been isolated and characterized from *Breonadia salicina*. Also isolated were three other coumarins and three triterpenes. Their NMR spectra were in agreement with literature reports thus confirming their identities.

Conflict of interest

The authors declare no conflict of interest.

Authors’ Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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